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Serotonin and Lysergic Acid Diethylamide Binding in Rat Brain Membranes: Relationship to Postsynaptic Serotonin Receptors

JAMES P. BENNETT, JR.,[1] AND SOLOMON H. SNYDER[2]

Departments of Pharmacology and Experimental Therapeutics and of Psychiatry and Behavioral Sciences, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

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SUMMARY

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[^{3}H]Serotonin (5-HT) binds to membrane preparations of rat brain in a saturable fashion and with substrate specificity and regional variations consistent with its binding to the postsynaptic serotonin receptor. The dissociation constant for [^{3}H]5-HT binding is about 8 nM, and the total number of 5-HT binding sites in the brain is 16 pmoles/g of tissue, wet weight. There is considerable structural specificity in the affinity of various tryptamines for the [^{3}H]5-HT binding sites, with a crucial role played by the 5-hydroxy substituent. *d*-[^{3}H]Lysergic acid diethylamide (LSD) binding sites have substrate specificity requirements similar to the [^{3}H]5-HT binding sites, but the 5-hydroxy substituent is less critical. 5-HT and related agonists have about 100 times more affinity for 5-HT than LSD binding sites, while classical 5-HT antagonists have 4-100 times greater affinity for LSD binding sites. LSD itself has a similar affinity for 5-HT and LSD binding sites. Raphe lesions which result in degeneration of 5-HT neurons do not lower [^{3}H]5-HT binding, indicating that binding does not take place to presynaptic 5-HT neurons. Regional variations in serotonin and LSD binding are fairly similar. Highest binding occurs in the corpus striatum, hippocampus, and cerebral cortex, with lowest binding in the cerebellum. The ontogeny of 5-HT and LSD binding sites is nearly identical and does not appear to depend on functionally intact presynaptic 5-HT neuronal input.

INTRODUCTION

Although an abundance of indirect pharmacological and neurochemical evidence has suggested for many years that 5-HT[3] is a neurotransmitter in the brain, only recently has direct neurophysiological investigation demonstrated postsynaptic inhibitory actions of 5-HT on nerve cells receiving an identified 5-HT input (1, 2). Recently, stereospecific binding of *d*-[^{3}H]LSD of high specific activity to brain membrane fragments has been observed, with characteristics resembling the physiological postsynaptic 5-HT receptor (3-6). However, in neurophysiological studies,

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[3] The abbreviations used are: 5-HT, 5-hydroxytryptamine (serotonin); LSD, lysergic acid diethylamide; TPCK, L-(1-tosylamido-2-phenyl)ethyl chloromethyl ketone; CPA, *p*-chlorophenylalanine; 5,7-DHT, 5,7-dihydroxytryptamine.

LSD is only a weak agonist at postsynaptic 5-HT receptors (1), whereas it is extremely potent as a 5-HT agonist in slowing the firing of 5-HT cells in the raphe nuclei, an effect presumably accounting for the psychedelic actions of the drug (7–9). In immature rat brain, 5-HT-stimulated adenylate cyclase is inhibited stereospecifically but incompletely by *d*-LSD (10). Recently, we reported binding of [^{3}H]5-HT to brain membranes which appears to be associated with postsynaptic 5-HT receptors (11). In the present study we compare properties of the binding of 5-HT and LSD to membrane fractions from rat brain.

MATERIALS AND METHODS

Adult male Sprague-Dawley rats were killed by decapitation, and their brains were rapidly removed and dissected on dental wax over ice. For routine binding studies, the regions were homogenized in 40 volumes of ice-cold 0.05 M Tris-HCl buffer (pH 7.4 at 37°), using a Brinkmann Polytron (setting 5, 20 sec), and centrifuged at 50,000 × *g* for 10 min, and the pellet was washed once by resuspension in cold Tris buffer. The washed pellet was resuspended in 100 volumes of 0.05 M Tris-HCl buffer containing 0.1% *l*-ascorbic acid and 10 μM pargyline (incubation buffer, pH 7.1–7.2 at 37°), incubated for 10 min at 37°, and cooled on ice, and 2-ml aliquots (20 mg of original tissue content) were incubated in triplicate at 37° after addition of tritiated and unlabeled ligands. For *d*-[^{3}H]LSD binding, 1 μM unlabeled *d*-LSD was included as a blank for nonspecific binding (5). For [^{3}H]5-HT binding, 10 μM unlabeled 5-HT was included as a blank (see Fig. 3 and RESULTS for details). Specific binding is defined as the difference between the total counts trapped on the filter in the absence of unlabeled ligand and the counts trapped in the presence of 1 μM unlabeled *d*-LSD or 10 μM unlabeled 5-HT. The samples were then rapidly vacuum-filtered through Whatman GF/B filters, and the incubation tubes and filter wells were rapidly rinsed with two 5-ml aliquots of ice-cold Tris buffer. Each filter well was then rinsed with an additional 5 ml of Tris buffer. Using this procedure, less than 0.1% of total added ^{3}H-labeled ligand was bound nonspecifically to the filters in the absence of tissue. The filters were then immersed in 12 ml of Hydromix (Yorktown Research, New Hyde Park, N. Y.), and radioactivity extracted overnight at 4° was quantitated in either a Packard 3385 or Searle Iso-Cap liquid scintillation spectrometer at a tritium counting efficiency of 44–46%.

For subcellular fractionation studies, brain tissue was homogenized in 10 volumes of ice-cold 0.32 M sucrose, using a Potter-Elvehjem glass homogenizer with a motor-driven Teflon pestle. The homogenate was centrifuged (4°) at 1000 × *g* for 10 min, yielding a crude nuclear pellet (P1) which was washed once with 10 volumes of cold 0.32 M sucrose and centrifuged again at 1000 × *g* for 10 min. Combined supernatants were centrifuged at 27,500 × *g* for 10 min to yield a crude mitochondrial pellet (P2); the supernatant was centrifuged at 100,000 × *g* for 1 hr, yielding a crude microsomal pellet (P3). Subfractionations of intact and lysed P2 pellets were performed with slight modifications of the procedures of Gray and Whittaker (12) and Whittaker *et al.* (13), respectively. P2 pellets were subfractionated intact by resuspension in 10 volumes of 0.32 M sucrose, layering onto discontinuous 0.8/1.2 M sucrose gradients, and centrifugation at 100,000 × *g* for 1 hr. P2 pellets were osmotically lysed by resuspension in 5 volumes of cold H_2O, allowed to stand on ice for 30 min, and centrifuged at 10,000 × *g* for 20 min. Aliquots (10 ml) of the supernatant were layered onto discontinuous sucrose gradients of 1.2 M (5 ml), 1.0 M (5 ml), 0.8 M (5 ml), 0.6 M (5 ml), and 0.4 M (5 ml) and centrifuged at 100,000 × *g* for 2 hr. Material accumulating at the sucrose interfaces was carefully aspirated, diluted to 15 volumes with Tris buffer, disrupted with the Polytron, and centrifuged at 50,000 × *g* for 10 min. The pellets were resuspended in either 25 volumes (intact P2) or 8 volumes (lysed P2) of incubation buffer and assayed as previously described.

For measuring [^{3}H]5-HT uptake, 100-μl aliquots of tissue homogenate or subcellular fractions equivalent to 10 mg of tissue wet weight in 0.32 M sucrose were added in triplicate to 2 ml of Krebs-Ringer-Tris

medium containing 0.1% *l*-ascorbic acid, 11 mM glucose, and 10 μM pargyline in the presence and absence of 100 nM Lilly 110140 (3-[*p*-trifluoromethylphenoxy]-*N*-methyl-3-phenylpropylamine hydrochloride), a specific inhibitor of high-affinity synaptosomal 5-HT transport (14). The samples were incubated for 6 min at 37°, returned to ice, and centrifuged at 27,500 × *g* for 10 min. The resulting pellets were washed once with cold Krebs buffer and centrifuged at 50,000 × *g* for 10 min, and pellet radioactivity was extracted into 1 ml of Protosol solubilizer (New England Nuclear Corporation). Ten milliliters of "LSC" complete scintillation mixture (Yorktown Research) were added, and accumulated [^{3}H]5-HT was assayed at a tritium counting efficiency of 30%.

Rat midbrain raphe nuclei were electrolytically lesioned as described previously (15). Control lesions were placed 1–2 mm lateral to the median raphe area.

d-[^{3}H]LSD (16–20 Ci/mmole) was obtained from New England Nuclear Corporation and Amersham-Searle. [^{3}H]5-HT (16–17 Ci/mmole) was obtained from Amersham-Searle. Unlabeled 5-HT and tryptamines were obtained from Regis Chemical Company. Trypsin-TPCK (203 units/mg), α-chymotrypsin (45 units/mg), and neuraminidase (0.54 unit/mg) were obtained from Worthington Biochemicals; phospholipase A (bee venom, 1000 units/mg), from Calbiochem; and phospholipase D (19 units/mg), from Sigma Chemical Company. Unlabeled *d*-LSD, LSD analogues, psilocin, and psilocybin were supplied by the NIMH-FDA Committee on Scheduled Substances. The following drugs were generous gifts: α- and β-flupenthixol (Dr. P. Seeman), (+)- and (−)-butaclamol (Ayerst Laboratories), and mianserine, cyproheptadine, methiothepin, and methysergide (Dr. J. Fernstrom). Clinically used phenothiazines, antidepressants, and related drugs were gifts from their pharmaceutical companies of origin.

RESULTS

Tissue linearity, pH dependence, time course, and effects of ions on specific [^{3}H]5-HT binding. Under standard incubation conditions the specific binding of [^{3}H]5-HT is linear between 5 and 40 mg of original wet weight of tissue per 2-ml incubation volume. All experiments were conducted routinely within this linear range. Specific [^{3}H]5-HT binding is maximal over a broad range of pH, with essentially the same amount of binding between pH 6.7 and 7.5. At 37 ° binding occurs rapidly, reaches half-maximal values at about 1 min, and attains equilibrium by 4 min (Fig. 1A). Binding is completely reversible, since when excess unlabeled 5-HT (10 μM) is added to incubation media which have reached equilibrium with [^{3}H]5-HT, the bound [^{3}H]5-HT has completely dissociated in 4 min at 37° (Fig. 1B). The kinetics of binding is slower at lower temperatures. At 25° association equilibrium requires about 20 min; at 4° equilibrium is not attained until 120 min after the addition of [^{3}H]5-HT.

The half-life for dissociation of bound [^{3}H]5-HT, determined by adding 10 μM nonradioactive 5-HT to preparations which have come to equilibrium with [^{3}H]5-HT, is about 3.5 min at 25° and 2 hr at 4° (Fig. 1B). At 25° the dissociation of [^{3}H]5-HT clearly is monophasic when plotted on a semilogarithmic scale, with a dissociation rate constant (k_{-1}) of 2.9×10^{-3} sec^{-1}. The bimolecular rate constant for association (k_1) of [^{3}H]5-HT at 25° is about 2.4×10^5 M^{-1} sec^{-1}. The dissociation constant (K_D) determined from the ratio k_{-1}/k_1 is 12 nM. Additionof 4 mM calcium chloride results in a consistent 20% increase in specific [^{3}H]5-HT binding and no change in specific *d*-[^{3}H]LSD binding; accordingly, routine incubations included 4 mM CaCl. The binding of [^{3}H]5-HT and *d*-[^{3}H]LSD is unaffected by up to 10 mM magnesium chloride. At 120 mM, NaCl, KCl, and choline chloride each inhibit the binding of [^{3}H]5-HT and *d*-[^{3}H]LSD by about 30%.

Thin-layer chromatography of bound [^{3}H]serotonin. *d*-[^{3}H]LSD bound to rat brain membranes is apparently unaltered after elution from the membranes (5). To determine whether [^{3}H]5-HT is altered after binding under the same conditions, rat forebrain membranes were incubated with 20 nM [^{3}H]5-HT in the presence and absence of 10 μM unlabeled 5-HT. The

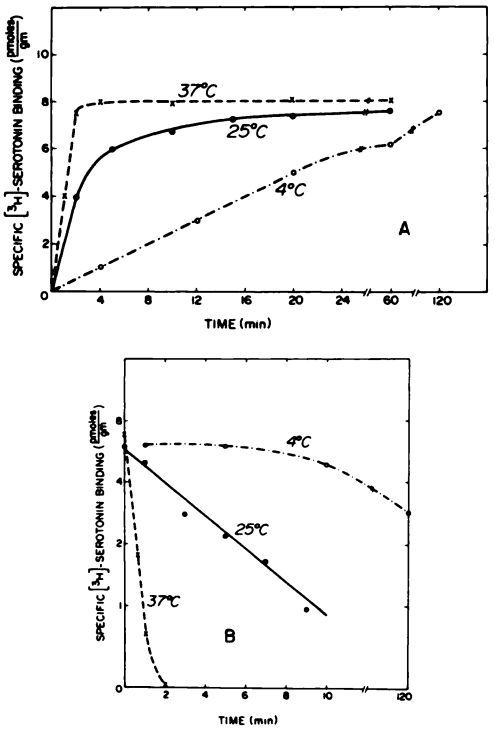

FIG. 1. *Association (A) and dissociation (B) of [³H]serotonin binding*

A. Specific binding of 7 nM [³H]5-HT to cerebral cortex membranes was assayed as described in MATERIALS AND METHODS at the indicated temperatures and after incubation for various periods. The experiment was replicated twice.

B. [³H]5-HT (7 nM) was incubated with tissue for 10 min at 37°. The samples were then equilibrated to the indicated temperatures for 10 min, and 10 μM unlabeled 5-HT was added at various times before assay. The samples were incubated with unlabeled 5-HT for the indicated time intervals, and specific [³H]5-HT binding was assayed as described in MATERIALS AND METHODS. The experiment was replicated twice.

membranes were isolated by centrifugation at 4° and washed once with cold Tris buffer. Radioactivity was eluted from the membranes by adding 5 ml of 90% ethanol containing 1% *l*-ascorbic acid and incubating the suspensions at 37° for 20 min. The supernatants resulting after centrifugation at 4° were reduced in volume under N_2 and analyzed on cellulose thin-layer plates using 1-butanol–pyridine–water (1:1:1) or 1-butanol–acetic acid–water (12:3:5). "Unbound" [³H]5-HT was also subjected to these procedures, but omitting contact with membranes. Total and nonspecifically bound [³H]5-HT, "unbound" [³H]5-HT, and unlabeled 5-HT all chromatographed with identical R_f values in both solvent systems, suggesting that at least 90% of bound [³H]5-HT is unaltered.

Saturation of [³H]5-HT binding. At concentrations less than 10 nM [³H]5-HT, total [³H]5-HT bound to cerebral cortex membranes is about 2–3 times the nonspecific [³H]5-HT binding determined in the presence of 10 μM nonradioactive 5-HT (Fig. 2A). The 10 μM 5-HT concentration was utilized as a "blank," becaue displacement curves indicated a plateau in displacement of [³H]5-HT by nonradioactive

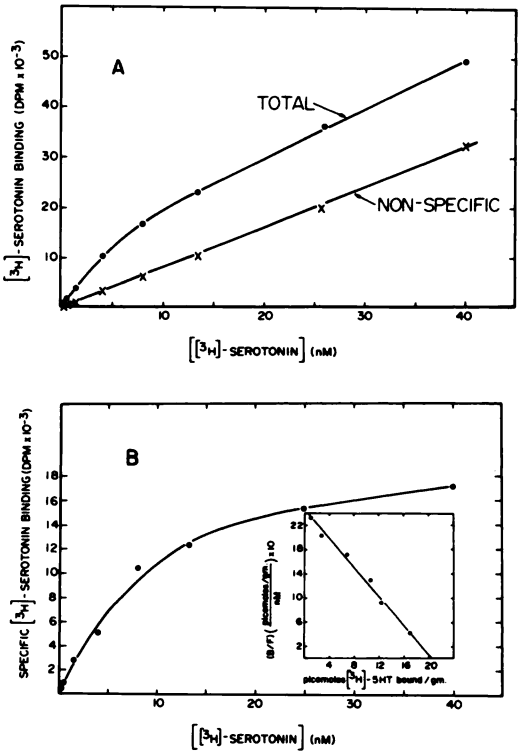

FIG. 2. *Saturation of [³H]serotonin binding*

A. Increasing concentrations of [³H]5-HT were incubated in the presence (nonspecific) and absence (total) of 10 μM unlabeled 5-HT, and binding to cerebral cortex membranes was assayed as described in MATERIALS AND METHODS.

B. Specific [³H]5-HT binding to cerebral cortex membranes was calculated by subtracting nonspecific binding from total binding. The inset presents the same data in a Scatchard plot. The experiment was replicated five times.

5-HT between 1.0 and 10 μM, which is similar to the maximal displacment of [^{3}H]5-HT binding by unlabeled *d*-LSD (Fig. 3A). At higher concentrations of [^{3}H]5-HT, total binding starts to saturate while nonspecific binding increases linearly, so that at 40 nM [^{3}H]5-HT total binding is only 40% greater than nonspecific binding (Fig. 2A). Specific [^{3}H]5-HT binding to cerebral cortex membranes has largely saturated by about 25 nM concentration, with half-maximal saturation at about 8 nM [^{3}H]5-HT (Fig. 2B). A Scatchard analysis of [^{3}H]5-HT binding indicates an apparent single population of binding sites, with a dissociation constant of 8 nM and a total receptor concentration of about 20 pmoles/g of original cerebral cortex tissue (Fig. 2B).

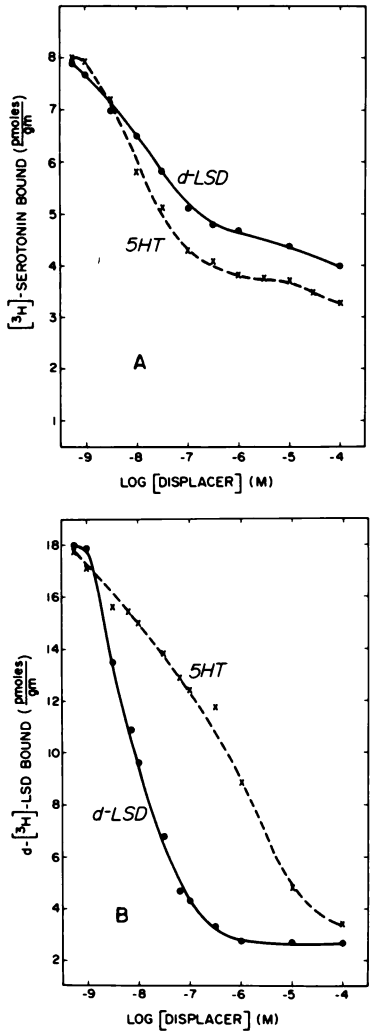

FIG. 3. *Displacement of [^{3}H]serotonin and d-[^{3}H]LSD binding by LSD and 5-HT*

A. [^{3}H]5-HT (7 nM) was incubated with increasing concentrations of unlabeled 5-HT and *d*-LSD, and binding was assayed as described in MATERIALS AND METHODS. The data represent total [^{3}H]5-HT binding. The experiment was replicated five times.

B. *d*-[^{3}H]LSD (3 nM) was incubated with increasing concentrations of unlabeled 5-HT and *d*-LSD, and binding was assayed as described in MATERIALS AND METHODS. The data represent total *d*-[^{3}H]LSD binding. The experiment was replicated six times.

In experiments in which [^{3}H]5-HT binding is inhibited by nonradioactive 5-HT, half-maximal inhibition occurs at about 10 nM concentration (Fig. 3A), similar to the saturation results obtained using only [^{3}H]5-HT. Unlabeled *d*-LSD inhibits [^{3}H]5-HT binding half-maximally at 10 nM, with inhibition by 10 μM *d*-LSD almost as complete as by 10 μM 5-HT.

Substrate specificity of [^{3}H]5-HT and [^{3}H]LSD binding. In confirmation of earlier studies (3-6), *d*-LSD inhibits *d*-[^{3}H]LSD binding with an ED_{50} value of about 6–10 nM, while the psychotropically inactive isomer *l*-LSD has about 1000 times less affinity for the LSD binding sites (Table 1). *d*-LSD has about the same affinity for [^{3}H]5-HT as for *d*-[^{3}H]LSD binding sites, with 50% inhibition at about 10 nM (Fig. 3), while *l*-LSD has 10,000 times less affinity than *d*-LSD for the [^{3}H]5-HT binding sites. *d*-Isolysergic acid amide and methysergide have similar affinities for both [^{3}H]5-HT and *d*-[^{3}H]LSD binding sites, about 1/10 that of *d*-LSD itself. *d*-Lysergic acid is less than 1% as potent as *d*-LSD in affecting both [^{3}H]5-HT and *d*-[^{3}H]LSD binding. 2-Bromo-LSD does differentiate between *d*-[^{3}H]LSD and [^{3}H]5-HT binding sites. In repeated experiments, 2-bromo-LSD had the same potency as *d*-LSD in inhibiting *d*-[^{3}H]LSD binding but was consistently about 1/10 as potent as *d*-LSD in inhibiting [^{3}H]5-HT binding.

Even more marked discrepancies between the 5-HT and LSD binding sites are evident in the influence of tryptamine derivatives. While 10 nM 5-HT reduces the binding of [^{3}H]5-HT 50%, 5-HT is about 100 times weaker in inhibiting *d*-[^{3}H]LSD binding. Similarly, bufotenine, the *N,N*-

TABLE 1

Displacement of specifically bound [^{3}H]serotonin and d-[^{3}H]LSD from rat cerebral cortex membranes

d-[^{3}H]LSD (3 nM) and [^{3}H]5-HT (7 nM) were incubated with cerebral cortex membranes and at least five concentrations of each drug and assayed as described in MATERIALS AND METHODS. Nonspecific binding occurring in the presence of 1 μM unlabeled *d*-LSD and 10 μM unlabeled 5-HT was subtracted from each of the respective determinations. The concentration of each drug giving half-maximal displacement of each ^{3}H-labeled ligand (IC_{50}) was determined by log probit analysis. Values are the means of three to six determinations, whose values varied less than 25%.

	IC_{50}	
	[^{3}H]5-HT	*d*-[^{3}H]LSD
	nM	*nM*
LSD analogues		
d-LSD	10	8
2-Bromo-LSD	100	10
d-Isolysergic acid amide	100	200
Methysergide	300	100
d-Lysergic acid	3,000	10,000
l-LSD	100,000	20,000
Tryptamines		
5-HT	10	1,000
5-Methoxytryptamine	50	1,000
Tryptamine	1,000	5,000
5-Hydroxy-*N,N*-dimethyltryptamine (bufotenine)	20	300
N,N-Dimethyltryptamine	2,000	2,000
4-O-Phosphoryl-*N,N*-dimethyltryptamine (psilocybin)	2,000	4,000
4-Hydroxy-*N,N*-dimethyltryptamine (psilocin)	1,000	1,000
5,6-Dihydroxytryptamine	600	7,000
5,7-Dihydroxytryptamine	>10,000	30,000
Anti-serotonin drugs		
Cyproheptadine	3,000	100
Methiothepin	300	60
Mianserine	10,000	100
Neurotransmitter candidates and analogues		
Dopamine	40,000	60,000
l-norepinephrine	300,000	10^6
l-isoproterenol	$>10^6$	$>10^6$
Acetylcholine, L-glutamic and L-aspartic acids, glycine, γ-aminobutyric acid	—[a]	—[a]
Other drugs		
5-Hydroxytryptophol	100,000	$>10^5$
5-Hydroxyindoleacetic acid	$>10^6$	$>10^5$
Fluphenazine	2,000	100
Chlorpromazine	5,000	100
Promethazine	>10,000	1,000
Haloperidol	>10,000	2,000
Atropine, morphine, Lilly 110140, Lilly 94939, cyclic AMP, dibutyryl cyclic AMP, cyclic GMP	—[a]	—[a]

[a] Less than 25% displacement at 100 μM.

dimethyl analogue of 5-HT, and 5-methoxytryptamine are 10–20 times more potent in inhibiting [^{3}H]5-HT than *d*-[^{3}H]LSD binding.

Various tryptamines differ in their relative influences on 5-HT and LSD binding. While tryptamine and *N,N*-dimethytryptamine are similar to 5-HT in their potency in inhibiting *d*-[^{3}H]LSD binding, they have only about 1% of the affinity of 5-HT for [^{3}H]5-HT binding sites. Thus, while LSD binding sites do not markedly differentiate among the various indole substituents, [^{3}H]5-HT binding sites display considerable structural specificity. The 5-hydroxy substitutent confers optimal activity, since 5-methoxytryptamine is only about one-fifth as potent as 5-HT itself, and tryptamine, which lacks a 5-hydroxy group, is only about 1% as potent as 5-HT. Psilocin, which differs from bufotenine only in possessing a 4-hydroxy instead of a 5-hydroxy group, is only 2% as potent as bufotenine in competing for [^{3}H]5-HT binding sites. 5,6-Dihydroxytryptamine and 5,7-dihydroxytryptamine are about 60 and more than 1000 times weaker, respectively, than 5-HT in inhibiting [^{3}H]5-HT binding.

While the agonist 5-HT has much more affinity for [^{3}H]5-HT than *d*-[^{3}H]LSD binding sites and *d*-LSD, which may be a mixed agonist-antagonist (16, 17), has similar affinities for LSD and 5-HT sites, certain classical 5-HT antagonists appear to have more affinity for LSD than 5-HT binding sites. Thus methysergide and methiothepin are 3–5 times more potent in competing for LSD than 5-HT binding sites. The 5-HT antagonist cyproheptadine is 30 times more potent an inhibitor of LSD than 5-HT binding, and mianserine dis-

plays about 100 times greater affinity for LSD than 5-HT sites.

The amine grouping in 5-HT is important for binding, since 5-hydroxyindoleacetic acid and 5-hydroxytryptophol have negligible affinity for either LSD or 5-HT binding sites. The catecholamines dopamine, norepinephrine, and isoproterenol are quite weak in competing for both LSD and 5-HT binding. The phenothiazines fluphenazine and chlorpromazine do inhibit the binding of LSD 50% at 100 nM concentration, about 20–50 times more effectively than they inhibit 5-HT binding. The equal potency of these two phenothiazines contrasts with the much greater potency of fluphenazine than of chlorpromazine in inhibiting the dopamine-sensitive adenylate cyclase which appears to be associated with the dopamine receptor (18–24). Promethazine and haloperidol are about $^1/_{10}$ as potent as fluphenazine and chlorpromazine in competing for LSD binding and fail to alter [^{3}H]5-HT binding even at 10 μM levels.

Subcellular distribution of d-[^{3}H]LSD and [^{3}H]5-HT binding. Approximately 60% and 70% of specific [^{3}H]5-HT and *d*-[^{3}H]LSD binding, respectively, localize to the crude mitochondrial (P2) fraction, known to contain many pinched-off nerve endings (synaptosomes) (Table 2). The highest specific activity for binding of both ligands in crude fractions is in the crude microsomal fraction (P3), which contains synaptosomes as well as membranes (12, 27). Subfractionation of P2 reveals that about 60% of the binding of both ligands occurs in the synaptosomal band and about 15% in the myelin band, with less than 30% binding to the "free mitochondria" fraction. The subcellular distribution of [^{3}H]5-HT and *d*-[^{3}H]LSD binding differs from that of high-affinity synaptosomal transport of [^{3}H]5-HT. High-affinity [^{3}H]5-HT transport localizes more than LSD or 5-HT binding to the crude mitochondrial (P2) and synaptosomal subfraction. When the P2 pellet is hypotonically lysed, greater than 80% of the total specific binding of both ligands remains in the 10,000 × *g* (20 min) supernatant. When this supernatant is subfractionated on discontinuous sucrose gradients, about one-half of the total specific binding of both ligands localizes in the 0.6/0.8 M and 0.8/1.0 M sucrose interfaces, which consist of synaptosome ghosts and membrane fragments (13, 28). One-fifth to one-fourth of the total specific binding for both ligands is found in the 1.0/1.2 M sucrose interface, which consists primarily of damaged synaptosomes. Very little binding occurs to free mitochondria at the bottom of the gradient.

Effects of protein-modifying reagents, enzymatic treatments, and detergents on specific d-[^{3}H]LSD and [^{3}H]5-HT binding. To determine which membrane components and functional groups might be involved in high-affinity *d*-[^{3}H]LSD and [^{3}H]5-HT binding, we subjected rat cerebral cortex membranes to treatment with a variety of protein-modifying reagents, enzymes, and detergents and then utilized the treated membranes in standard binding studies (Table 3). Both *d*-[^{3}H]LSD and [^{3}H]5-HT binding are sensitive to low concentrations of reagents which modify sulfhydryl groups and are reduced similarly by prior treatment with *N*-ethylmaleimide and 5,5′-dithiobis(2-nitrobenzoic acid), and somewhat less by *N*-bromosuccinimide. Prior treatment with 2-hydroxy-5-nitrobenzyl bromide, which alters tryptophan residues, reduces binding only slightly or not at all at a concentration (10 mM) which lowers opiate agonist binding by 53% (29). The carboxyl group reagent 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (1 mM) decreases *d*-[^{3}H]LSD binding by about 50% and [^{3}H]5-HT binding by about 75%.

The binding sites for *d*-[^{3}H]LSD and [^{3}H]5-HT appear to involve protein structure, as the binding of both ligands is reduced by prior treatment with low concentrations of trypsin-TPCK and α-chymotrypsin. [^{3}H]5-HT binding is somewhat more sensitive than LSD binding to previous treatment with α-chymotrypsin. Prior treatment with phospholipase A, which hydrolyzes fatty acids from phosphoglycerides, drastically reduces the specific binding of both ligands. In contrast, prior treatment with phospholipase D, which hydrolyzes the polar group from

TABLE 2

Subcellular distribution of specific d-[³H]LSD and [³H]serotonin binding in rat cerebral cortex

d-[³H]LSD (3 nM) and [³H]5-HT (7 nM) were incubated with various subcellular fractions, prepared and assayed as described in MATERIALS AND METHODS. [³H]5-HT uptake into homogenates was assayed as described in the text; radioactivity present after incubation with 100 nM Lilly 110140 was subtracted as a blank value. Monoamine oxidase and succinic acid dehydrogenase activities were determined according to the methods of Wurtman and Axelrod (25) and Davis and Bloom (26), respectively. Values are the averages from two separate experiments, whose results differed less than 20%.

Subcellular fractions	Specific binding		Percentage of total				
	[³H]LSD	[³H]5-HT	Binding		[³H]5-HT uptake	Monoamine oxidase	Succinate dehydrogenase
			[³H]LSD	[³H]5-HT			
	fmoles/mg protein						
Unlysed homogenate							
Whole homogenate	210	160					
Crude nuclear (P1)	160	63	17	21	12	16	
Crude mitochondrial (P2)	210	70	72	62	87	75	
Crude microsomal (P3)	520	170	12	17	1	9	
Subfractionation of P2							
0.3/0.8M: myelin, etc.	470	240	12	15	1	2	2
0.8/1.2M: synaptosomes	370	180	59	58	97	46	47
>1.2M: mitochondria	91	34	29	27	2	52	51
Lysed P2							
10,000 × *g* pellet	230	140	16	13		49	57
10,000 × *g* supernatant	420	220	84	87		51	43
0/0.4 M: vesicles, microsomes	0	0	0	7		0	0
0.4/0.6 M: myelin, microsomes, vesicles	260	240	11	15		0	0
0.6/0.8 M: synaptosome ghosts, myelin, nonvesicular membrane fragments	375	320	13	14		0	0
0.8/1.0 M: synaptosome ghosts, membrane fragments	720	640	33	31		3	0
1.0/1.2 M: damaged synaptosomes	660	570	24	20		52	53
Pellet: mitochondria, shrunken synaptosomes	130	110	19	13		45	47

phosphoglycerides, in repeated experiments increases the specific binding of both ligands. Prior treatment with neuraminidase, which removes sialic acid residues, also increases the binding of *d*-[³H]LSD and [³H]5-HT. Conceivably phospholipase D and neuraminidase remove membrane moieties which normally mask receptor binding. Unmasking of "buried" insulin receptors has been observed with phospholipases (30).

The binding sites for *d*-[³H]LSD and [³H]5-HT are affected by low concentrations of the nonionic detergent Triton X-100 and the anionic detergent sodium deoxycholate. The nonionic detergent Tween-80 is much less effective in reducing ligand binding.

Regional localization of 5-HT and LSD binding. The possibility that LSD and 5-HT bind to related sites is supported by the general similarity in the regional distribution of their binding (Table 4). The apparent dissociation constants and total number of receptor sites in four different rat brain regions were determined by obtaining saturation curves of increasing amounts of *d*-[³H]LSD and [³H]5-HT in the four brain regions with highest binding (Table 3). When studied at equal concen-

TABLE 3

Effects of protein-modifying reagents, enzymatic treatments, and detergents on specific d-[³H]LSD and [³H]serotonin binding to rat cerebral cortex membranes

Rat cerebral cortex membranes were treated for 20 min at 37° (protein-modifying reagents and enzymes) or at 4° or room temperature (detergents), isolated by centrifugation, and washed once with 40 volumes of cold Tris buffer. The specific binding of 3 nM *d*-[³H]LSD and 7 nM [³H]5-HT to treated and control membranes was then determined as described in MATERIALS AND METHODS. The incubation medium for phospholipase A treatment contained 5 mM $CaCl_2$. Values are the means of two independent determinations, which varied less than 5%.

Substance tested	Specific binding	
	d-[³H]LSD	[³H]5-HT
	% control	
Protein-modifying reagents		
N-Ethylmaleimide, 1 mM	57	38
5,5'-Dithiobis(2-nitrobenzoic acid), 5 mM	65	59
N-Bromosuccinimide, 10 μM	72	76
2-Hydroxy-5-nitrobenzyl bromide, 10 mM	90	100
1-Ethyl-3-(3-dimethylamino-propyl)carbodiimide, 1 mM	49	24
Enzymes		
Trypsin-TPCK, 2.0 units/ml	52	52
α-Chymotrypsin, 0.45 unit/ml	76	49
Phospholipase A, 1.0 unit/ml	4.0	11
Phospholipase D, 1.9 units/ml	133	122
Neuraminidase, 0.03 unit/ml	137	145
Detergents		
0.1% Triton X-100		
4°	2.6	6.3
Room temperature	0.4	1.0
0.5% Sodium deoxycholae		
4°	0.9	9.1
Room temperature	0.9	2.5
1% Tween 80		
4°	57	75
Room temperature	36	45

trations of both ligands, all regions display 2.5–3 times as much *d*-[³H]LSD binding as [³H]5-HT binding (Table 4). For both ligands cerebellar binding is the lowest, equaling about 10–15% of binding observed in the highest regions, and the cerebral cortex, corpus striatum, and hippocampus are the areas of highest binding. Comparison of the apparent dissociation constants (K_D) and maximal numbers of binding sites for *d*-[³H]LSD and [³H]5-HT in the four highest regions shows that *d*-[³H]LSD has higher affinity and more binding sites than [³H]5-HT. The ratio of the numbers of LSD to 5-HT sites is about 2 for the brain regions examined.

Previously it had been shown that *d*-[³H]LSD binding does not occur to membranes of 5-HT neurons, since binding is unaffected after lesions of 5-HT cell bodies in the midbrain dorsal and median raphe nuclei which cause nearly complete degeneration of 5-HT neurons and nerve terminals in the forebrain (3–5, 15). In the present study we evaluated the influence of raphe lesions on [³H]5-HT and *d*-[³H]LSD binding by rat forebrain membranes at two time points after lesioning. Experimental animals received electrolytic lesions of the midbrain raphe nuclei or of control areas slightly lateral to the raphe. The accumulation of [³H]5-HT (5 nM) by crude synaptosomal preparations of the forebrain is 48 ± 6 pmoles/g, wet weight, for control animals and 8.3 ± 1.0 pmoles/g, wet weight, for lesioned animals 2 weeks after lesioning, indicating an 83% reduction in synaptosomal 5-HT uptake in lesioned animals.

When increasing concentrations of [³H]5-HT are incubated simultaneously with forebrain membranes of raphe-lesioned (2 weeks after lesions) and control rats, there appears to be a 2-fold decrease in the apparent K_D for [³H]5-HT in the lesioned forebrains, with no change in the maximum number of detectable binding sites (Fig. 4A). Similar saturation experiments for *d*-[³H]LSD binding in the same animals revealed a slight decrease in the apparent K_D for *d*-[³H]LSD in the lesioned forebrain membranes, with no change in the maximum number of detectable binding sites (Fig. 4B).

In rats with similar, nearly total lesions of the midbrain raphe nuclei, Kuhar *et al.* (15) observed significant reductions in total forebrain 5-HT levels in addition to the reductions in high-affinity synaptosomal

TABLE 4

Regional localization of d-[³H]LSD and [³H]serotonin binding in rat brain

The specific binding of various concentrations of *d*-[³H]LSD and [³H]5-HT to rat brain membranes from different regions was assayed as described in MATERIALS AND METHODS. The equilibrium dissociation constant (K_D) and maximal number of binding sites (B_{max}) were estimated from Scatchard plots. Values are averages of two separate determinations, which varied less than 25%.

Region	Specific binding		*d*-[³H]LSD		[³H]5-HT		R^a
	d-[³H]LSD 7.0 nM	[³H]5-HT, 7.0 nM	K_D	B_{max}	K_D	B_{max}	
	pmoles/g		*nM*	*pmoles/g*	*nM*	*pmoles/g*	
Cerebral cortex	27.8	7.82	5.7	39	9.6	18	2.2
Corpus striatum	31.0	9.74	6.1	48	10	24	2.0
Thalamus	16.7	6.72	6.8	28	11	19	1.5
Hippocampus	27.8	10.4	5.1	38	8.3	25	1.5
Midbrain	9.85	3.93	7.0	24	15	12	2.0
Brain stem	7.34	2.19					
Cerebellum	3.44	1.44					

[a] R is the ratio of the B_{max} of *d*-[³H]LSD to the B_{max} of [³H]5-HT.

[³H]5-HT uptake. We reasoned that the apparent increased affinity for [³H]5-HT in raphe-lesioned forebrain membranes might simply reflect less competition for binding by the lower levels of endogenous 5-HT not removed by the standard membrane washing procedure. In this case pharmacological depletion of 5-HT should also increase [³H]5-HT binding. Accordingly, we compared the effects on [³H]5-HT and *d*-[³H]LSD binding of raphe lesions and systemic treatment with either reserpine or *p*-chlorophenylalanine, which can deplete endogenous brain 5-HT to approxi-

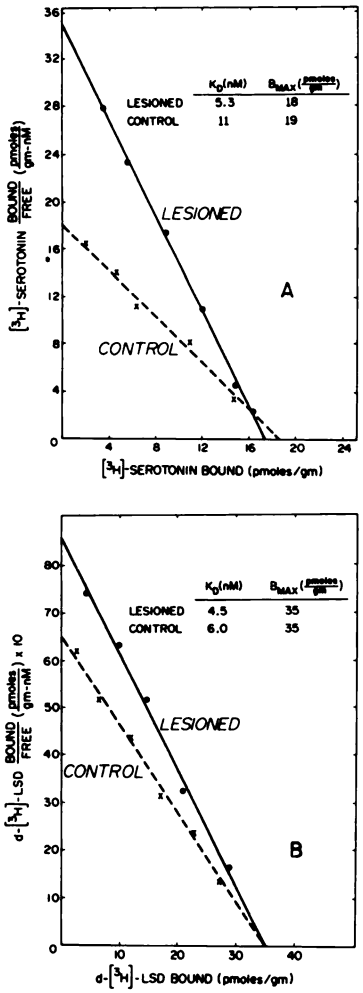

FIG. 4. *Saturation of [³H]serotonin and d-[³H]LSD binding to forebrain membranes of raphe-lesioned and control rats.*

A. Specific binding of increasing concentrations of [³H]5-HT to raphe-lesioned and control forebrain membrane was assayed as described in MATERIALS AND METHODS. The data are presented in a Scatchard plot, from which the equilibrium dissociation constants (K_D) and maximal number of binding sites (B_{max}) were determined. Data are averages from two separate experiments, which varied less than 25%.

B. Specific binding of increasing concentrations of *d*-[³H]LSD to the same forebrain membranes used in Fig. 4A was assayed as described in MATERIALS AND METHODS. The data are also presented in a Scatchard plot. Differences in K_D values for 5-HT and *d*-LSD between control and lesioned rats were the same in three repeated experiments. Data are averages from two separate experiments, which varied less than 25%.

mately the same levels as raphe lesions (31, 32).

One or two weeks after lesion placement, the specific binding of [^{3}H]5-HT (1–15 nM) to forebrain membranes of raphe-lesioned rats is elevated 40–60% over controls (Table 5). In forebrain membranes 2 weeks after raphe lesion placments, specific *d*-[^{3}H]LSD binding is increased 10–20% only at low (1–4 nM) *d*-[^{3}H]LSD.

The failure of 5-HT and LSD binding to decline after these lesions indicates that binding is not presynaptic. The enhanced binding might reflect synthesis of new binding sites, conceivably associated with receptor supersensitivity, or a depletion of endogenous 5-HT bound to receptors. To distinguish between these alternatives we evaluated the influence of 5-HT-depleting drugs.

After 1 or 2 weeks of treatment, reserpine (5 mg/kg intraperitoneally every 48 hr) or CPA (100 mg/kg intraperitoneally every 24 hr) elicited 20–300% increases in specific [^{3}H]5-HT binding in all brain regions except the cerebellum (Table 5). We observed smaller increases in specific *d*-[^{3}H]LSD binding (0–50%) in these brain regions from drug-treated rats (Table 5). At earlier times (6–24 hr) after reserpine (5 mg/kg intraperitoneally) or CPA (300 mg/kg intraperitoneally) we also detected 20–30% elevations in the binding of [^{3}H]5-HT, but not of *d*-[^{3}H]LSD, to forebrain mem-

TABLE 5

Effects of raphe lesions and reserpine or p-chlorophenylalanine treatments on specific binding of d-[^{3}H]LSD and [^{3}H]serotonin to rat brain membranes

Electrolytic lesions of the midbrain raphe nuclei were performed as previously described (15). Nonlesioned rats were treated with either reserpine (5 mg/kg intraperitoneally) for 6 and 24 hr and every 48 hr for 1 and 2 weeks, or with *p*-chlorophenylalanine, 300 mg/kg intraperitoneally for 24 hr and 100 mg/kg intraperitoneally every 24 hr for 1 and 2 weeks. Specific binding of various concentrations of *d*-[^{3}H]LSD and [^{3}H]5-HT to membranes from different brain regions of drug-treated rats and NaCl-treated controls was assayed as described in MATERIALS AND METHODS. The values given are the averages of two separate determinations varying less than 20%, with four to six rats utilized in each determination.

Region and treatment	Time of treatment	Increase over control binding		Region and treatment	Time of treatment	Increase over control binding	
		d-[^{3}H]LSD	[^{3}H]5-HT			*d*-[^{3}H]LSD	[^{3}H]5-HT
		%	%			%	%
Whole forebrain				Hippocampus			
Lesioned	1 wk	10	43	Reserpine	1 wk	4	27
	2 wk	20	64		2 wk	15	34
Reserpine	6 hr	0	30	CPA	1 wk	22	44
	24 hr	0	17		2 wk	22	45
CPA (300 mg/kg)	24 hr	0	22	Midbrain			
Cerebral cortex				Reserpine	1 wk	25	27
Reserpine	1 wk	20	50		2 wk	18	53
	2 wk	8	54	CPA	1 wk	56	75
CPA	1 wk	9	42		2 wk	52	62
	2 wk	26	44	Brain stem			
Striatum				Reserpine	1 wk	0	140
Reserpine	1 wk	29	30		2 wk	8	110
	2 wk	20	21	CPA	1 wk	34	290
CPA	1 wk	26	54		2 wk	51	280
	2 wk	34	81	Cerebellum			
Thalamus-hypothalamus				Reserpine	1 wk	0	0
					2 wk	0	0
Reserpine	1 wk	18	32	CPA	1 wk	0	19
	2 wk	0	43		2 wk	15	0
CPA	1 wk	18	64				
	2 wk	2	56				

branes. The enhanced binding observed at short intervals after 5-HT depletion would seem to result from depletion of endogenous 5-HT bound to receptors rather than from a proliferation of "supersensitive" receptors, which should display a longer time course. This suggests that lesion-induced enhancement of binding also results from reduced competition by endogenous 5-HT.

Effects of isomers of butaclamol and flupenthixol, and of dopamine, on specific [³H]5-HT and d-[³H]LSD binding to regions of rat brain. Several recent reports have presented behavioral (33) and biochemical (34–36) evidence suggesting that *d*-LSD may function as a dopamine agonist in rat striatum. Conceivably, stereospecific *d*-[³H]LSD binding might involve dopamine as well as 5-HT receptors.

(+)-Butaclamol and α-flupenthixol are 300–1000- and 1000-fold more potent, respectively, than their corresponding (−) and β isomers, both behaviorally as neuroleptics (37) and biochemically as inhibitors of the dopamine-sensitive adenylate cyclase of corpus striatum (23) and olfactory tubercle (22). To evaluate possible binding of *d*-LSD to dopamine receptors, we studied the effects of (+)- and (−)-butaclamol and α- and β-flupenthixol and dopamine on specific *d*-[³H]LSD and [³H]5-HT binding to membranes of rat cerebral cortex, corpus striatum, and hippocampus.

Dopamine requires concentrations of 30–60 μM to lower *d*-[³H]LSD or [³H]5-HT binding 50%. In all regions dopamine is 500–3000 times weaker than 5-HT in competing for LSD binding (Fig. 5 and Table 6). Butaclamol and flupenthixol isomers

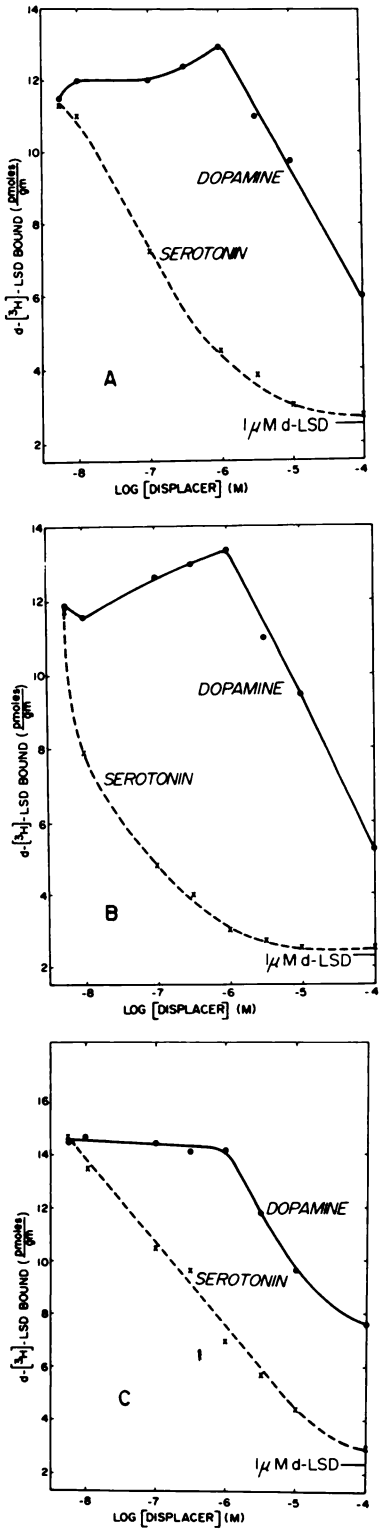

FIG. 5. *Displacement of d-[³H]-LSD binding by 5-HT and dopamine*

The binding of 3 nM *d*-[³H]LSD to membranes from three brain regions in the presence of various concentrations of unlabeled 5-HT and dopamine was assayed as described in MATERIALS AND METHODS. The data represent total *d*-[³H]LSD binding. The amount of *d*-[³H]LSD bound in each region in the presence of 1 μM unlabeled *d*-LSD is indicated in the lower right-hand corner of each figure (A, cerebral cortex; B, hippocampus; C, corpus striatum). The experiment was replicated three times.

TABLE 6

Displacement of specifically bound d-[³H]LSD and [³H]serotonin by neuroleptic isomers and unlabeled d-LSD, serotonin, and dopamine

Specific binding of 3 mM *d*-[³H]LSD and 7 nM [³H]5-HT to membranes of different brain regions was assayed as described in MATERIALS AND METHODS. At least five different concentrations of each unlabeled drug were assayed, and concentrations which reduced binding 50% (IC_{50}) were determined by log probit analysis. Values given are the averages from two or three separate determinations, which varied less than 15%.

Region	IC_{50} values						
	(+)-Butaclamol	(−)-Butaclamol	α-Flupenthixol	β-Flupenthixol	*d*-LSD	5-HT	Dopamine
	nM	*nM*	*nM*	*nM*	*nM*	*nM*	*nM*
[³H]LSD binding							
Cerebral cortex	50	7,000	100	6,000	8	500	60,000
Striatum	30	100,000	35	400	6	700	30,000
Hippocampus	300	50,000	2,000	15,000	7	16	45,000
[³H]5-HT binding							
Cerebral cortex	1,000	8,000	4,000	60,000	10	10	40,000
Striatum	30,000	150,000	10,000	100,000	8	11	50,000
Hippocampus	1,300	90,000	4,000	60,000	8	10	50,000

display marked stereospecificity for 5-HT as well as for LSD binding sites. The displacement of *d*-[³H]LSD from hippocampal membranes is considerably less sensitive to (+)-butaclamol and α-flupenthixol than in cerebral cortex or corpus striatum. 5-HT is also 30–40 times more potent in displacing *d*-[³H]LSD in the hippocampus than in cerebral cortex or corpus striatum (Table 6).

Developmental patterns of d-[³H]LSD and [³H]5-HT binding and effects of 5,7-dihydroxytryptamine injection. The binding of *d*-[³H]LSD and [³H]5-HT to rat cerebral cortex membranes shows marked similarities throughout early development. In experiments using increasing concentrations of *d*-[³H]LSD and [³H]5-HT with cerebral cortex membranes from rats of varying postnatal ages alongside adult cortex membranes, the apparent affinity constants for both ligands did not change substantially during development (Table 7). In contrast, the apparent maximal numbers of high-affinity LSD and 5-HT binding sites undergo similar augmentations in the course of development (Table 7). The numbers of LSD and 5-HT binding sites are only 7% of adult values at 1 day of postnatal age, increase to 31% of adult levels at 1 week, to 74–78% of adult levels at 2 weeks, and to adult levels by 3 weeks. Additionally, the Ed_{50} values for displacement of specifically bound *d*-[³H]LSD and [³H]5-HT by unlabeled *d*-LSD, 2-bromo-

TABLE 7

Developmental patterns of d-[³H]LSD and [³H]serotonin binding to rat cerebral cortex

Specific binding of various concentrations of *d*-[³H]LSD and [³H]5-HT to cerebral cortex membranes from rats of different postnatal ages was assayed as described in MATERIALS AND METHODS, except that 2- and 4-fold higher tissue concentrations were used for the 1-week-old and 1-day-old samples, respectively. The equilibrium dissociation constants (K_D) and maximal number of binding sites (B_{max}) were estimated from Scatchard plots. Values are averages of two separate determinations, which varied less than 25%.

Postnatal age	*d*-[³H]LSD		[³H]5-HT		R^a
	K_D	B_{max}	K_D	B_{max}	
	nM	*pmoles/g*	*nM*	*pmoles/g*	
1 day	4.0	2.8	6.0	1.6	1.7
1 wk	5.6	12	5.1	5	2.4
2 wk	6.4	30	8.1	14	2.2
3 wk	5.0	37	7.3	18	2.1
Adult	6.0	37	8.0	18	2.1

[a] R is the ratio of the B_{max} of *d*-[³H]LSD to the B_{max} of [³H]5-HT.

LSD, and 5-HT at all ages studied are similar to adult values.

Baumgarten *et al.* (38) reported that the intracisternal injection of 40 μg of 5,7-dihydroxytryptamine to 2-day-old rats causes a rapid and long-lasting depletion of tryptophan hydroxylase activity in all forebrain areas. Tryptophan hydroxylase activity increases somewhat 12 days after injection but never exceeds 25% of control levels. We injected 40 μg of 5,7-DHT intracisternally into 2-day-old rats and 0.9% NaCl–ascorbic acid vehicle into littermate controls. At 2 weeks of postnatal age, 12 days after injection, the 5,7-DHT-treated rats weigh about one-half as much as controls, similar to the results of Baumgarten *et al.* (38), but average cerebral cortical weight is unchanged (360 mg for the 5,7-DHT-treated rats and 330 mg for controls).

High-affinity uptake of 5 nM [^{3}H]5-HT into crude synaptosomal preparations of cerebral cortex is about 40% of adult levels in control 2-week-old rats, and is blocked completely in both 2-week control and adult homogenates by the presence of 100 nM Lilly 110140, a potent, specific inhibitor of high-affinity synaptosomal 5-HT transport (14). Serotonin uptake is reduced to undetectable levels in 2-week-old rats treated 2 days postnatally with 5,7-DHT. In contrast to the effect of 5,7-DHT treatment on 5-HT uptake and tryptophan hydroxylase, high-affinity binding of [^{3}H]5-HT and *d*-[^{3}H]LSD to cerebral cortical membranes is not significantly altered. The apparent K_D values for both ligands are similar in 5,7-DHT-treated and control membranes (Table 8). The apparent maximum numbers of binding sites for both ligands are about 30% higher in control cortical membranes compared to 5,7-DHT-treated rat cortical membranes when expressed per gram, wet weight. When expressed per milligram of protein, the maximum number of binding sites is only about 10% higher in the control group. This suggests that development of serotonin receptors proceeds independently of serotonin neuronal development (39–43), resembling the development of central dopamine receptors (44).

The numbers of binding sites for both ligands are lower in the 2-week-old control membranes in the 5,7-DHT experiments than in developmental studies (Table 7), in which total membrane fractions were used. However, the ratio between the maximum number of LSD sites to 5-HT sites is still aprproximately 2. In addition, the specific binding of both ligands is displaced to the same extent in 5,7-DHT-treated 2-week membranes, control 2-week membranes, and adult membranes by unlabeled *d*-LSD, 5-HT, and 2-bromo-LSD.

DISCUSSION

LSD and 5-HT binding sites resemble each other closely. Of all known neurotransmitters, 5-HT has the highest affinity for both sites. In general, relative potencies of drugs are similar for the two binding sites, and regional and develop-

TABLE 8

Effects of 2-day postnatal treatment with intracisternal 5,7-dihydroxytryptamine on binding of d-[^{3}H]LSD and [^{3}H]serotonin to 2-week-old rat cerebral cortex

Two-day-old rat pups were injected intracisternally under aseptic conditions with 5,7-dihydroxytryptamine creatinine sulfate (40 μg of free base in 10 μl of vehicle) (38). At 14 days of age (12 days after injection) the animals were killed and specific binding of various concentrations of *d*-[^{3}H]LSD and [^{3}H]5-HT was determined as described in MATERIALS AND METHODS. The equilibrium dissociation constant (K_D) and maximal number of binding sites (B_{max}) were determined from Scatchard plots. Values are the means of two determinations varying less than 25%.

Treatment	*d*-[^{3}H]LSD			[^{3}H]5-HT		
	K_D	B_{max}		K_D	B_{max}	
	nM	*pmoles/g*	*fmoles/mg protein*	*nM*	*pmoles/g*	*fmoles/mg protein*
5,7-DHT	4.2	14	520	7.0	8.0	300
Vehicle	4.8	19	590	7.4	11.0	320

mental patterns are similar for both LSD and 5-HT binding. It is tempting to conclude that LSD and 5-HT both label the postsynaptic 5-HT receptor.

However, there are some striking differences in 5-HT and LSD binding, particularly in terms of substrate specificity. The "pure" agonist 5-HT has about 100 times more affinity for 5-HT than LSD binding sites, while a variety of classical 5-HT antagonists, such as methysergide, cyproheptadine, methiothepin, and mianserine, have 3–100 times greater affinity for LSD than 5-HT sites. These antagonists are potent in blocking the actions of 5-HT in various smooth muscle preparations (45, 46). In behavioral studies they appear to function as antagonists of 5-HT actions in the brain at postsynaptic receptors (47–50). However, in the limited neurophysiological investigations of their ability to antagonize 5-HT iontophoretically applied to cells receiving identified 5-HT neuronal input, they are at best weak 5-HT antagonists (2, 16, 17, 51–53).

d-LSD has similar affinities for 5-HT and LSD binding sites. Iontophoretic administration of LSD to cells with identified 5-HT input in the brain indicates that LSD is a weak agonist at postsynaptic 5-HT receptors. It behaves as a partial agonist, in that maximal effects from LSD are less than those from 5-HT and LSD does weakly antagonize iontophoretically administered 5-HT (1). Thus, at postsynaptic 5-HT receptors in the brain, LSD has properties consistent with those of a mixed agonist-antagonist.

If one assumes that the classical 5-HT antagonists employed here do in fact bind as antagonists at postsynaptic 5-HT receptors in the brain, the differential substrate specificity of [^{3}H]5-HT and *d*-[^{3}H]LSD binding sites accords well with a two-state model for synaptic receptor functioning (54–56). Biochemical studies of several neurotransmitter and drug receptors in the brain (11, 29, 56–59) support the existence of distinct agonist and antagonist states of receptors, which may be interconvertible to varying extents. We suggest that [^{3}H]5-HT binds to the agonist state of the 5-HT receptor while LSD, a mixed agonist-antagonist, binds to both agonist and antagonist states. Assuming that the interconversion of agonist and antagonist states of the receptor is restricted, one would predict that an agonist such as 5-HT would be substantially more potent in competing for the agonist than the antagonist state of the receptor, while the reverse situation would hold for pure antagonists. Mixed agonists-antagonists should bind with equal affinity to both agonist and antagonist states of the receptor. At postsynaptic 5-HT receptors in the brain, 2-bromo-LSD, which has about 10 times more affinity for LSD than 5-HT binding sites, might display more highly antagonistic properties than does LSD itself. Similarly, one would predict that tryptamine and *N*,*N*-dimethyltryptamine, with similar affinities for LSD and 5-HT binding sites, should be mixed agonist-antagonists, in contrast to the pure agonistic actions of 5-HT itself.

Both specific *d*-[^{3}H]LSD and [^{3}H]5-HT binding are displaced stereospecifically by LSD and butaclamol, and more potently by α- than β-flupenthixol in several regions of rat brain. Isomeric specificities for butaclamol and flupenthixol have been proposed as criteria for identifying the dopamine receptor (60). Their stereospecificity at 5-HT and LSD binding sites, which are insensitive to dopamine, emphasizes a need for caution in interpreting effects of these drugs.

There also appear to be subtle differences in the binding sites of LSD and 5-HT in different brain regions, as revealed by the different potencies of certain drugs in displacing these ligands. (+)-Butaclamol and α-flupenthixol are much weaker while 5-HT is more potent in displacing *d*-[^{3}H]LSD from hippocampal as compared to cerebral cortical or striatal membranes. Striatal [^{3}H]5-HT binding is less susceptible to displacement by (+)-butaclamol and α-flupenthixol than cerebral cortical or hippocampal [^{3}H]5-HT binding. These findings are consistent with recent observations that in the corpus striatum about 15–20% of *d*-[^{3}H]LSD binding involves dopamine receptors (61).

If there are energy constraints which limit the interconversion of agonist and antagonist states of the 5-HT receptor,

[^{3}H]5-HT should label only the agonist states while LSD would label both agonist and antagonist states. The number of high-affinity LSD binding sites should equal the total number of available 5-HT receptors, while the number of antagonist sites would equal the number of LSD minus the number of 5-HT binding sites. For several forebrain areas there are about twice as many LSD as 5-HT binding sites, so that there are about equal numbers of antagonist and agonist states of the 5-HT receptor. It is of course conceivable that 5-HT and LSD bind to completely different sites: that 5-HT binds to the 5-HT receptor while LSD binds to a site completely unrelated to the 5-HT receptor. Indeed, it is possible that 5-HT itself does not bind to the 5-HT receptor. However, the similarities in binding of 5-HT and of LSD are more compelling than the differences. The substrate selectivity, unique regional variations, nonpresynaptic localization, and nearly identical developmental patterns in the binding of 5-HT and LSD suggest that they do bind to the postsynaptic 5-HT receptor.

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